BIOCHEMISTRY

ISOLATION AND PARTIAL PURIFICATION OF SECONDARY ALCOHOL DEHYDROGENASE FROM *Bacillus sphaericus* 5d4, NRRL B-14865.

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Nocardia cholesterolicum NRRL 5767 is renowned for its strain stability, and its high yields in transforming oleic acid to10-hydroxystearic acid (10-HSA) with the highest yield (85% conversion). 10-HSA is an industrial useful product. However, a side-product 10-ketostearic acid (10-KSA) is also produced (10%) which complicates downstream separation and purification of these products. The conversion of oleic acid to 10-HSA and subsequently to 10-KSA is catalyzed by oleate hydratase and secondary alcohol dehydrogenase, respectively. Both enzymes are membrane bound proteins. The long term objective of this research is to create a microorganism that possesses only oleate hydratase, thus eliminating product purification and increasing the potential market for these agricultural products.

In our efforts to screen microorganisms that can convert agricultural commodities to value-added products, we isolated *Bacillus spharicus* 5d4 that can effectively convert oleic acid to 10-HSA and 10-KSA. The results suggested that this strain is an excellent source for isolation of secondary alcohol dehydrogenase (2^o-ADH). Purification and characterization of 2^o-ADH will enhance our goal to block the expression of a 2^o-ADH in *B. sphaericus* 5d4 and *NC* NRRL 5767 by genetic engineering approach. The 2^o-ADH has been partially purified from *B. sphaericus* 5d4 by ammonium sulfate fractionation and hydrophobic chromatography.

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